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Synthesis of an Inositol-Containing Trisaccharide Related to Insulin Signal Transduction

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ABSTRACT

A synthesis of D-mannosyl-(α1-4)-D-glucosaminyl-(α1-6)-D-*myo*-inositol-1:2-cyclic phosphate (**1**) from D-xylose, D-mannose, and D-glucal is reported. Compound **1** constitutes the terminal structure of the PI-PLC-released VSG membrane anchor of Trypanosomes and is structurally related to the inositol phoshate glycan implicated as an insulin second messenger in higher organisms.

For the last dozen years, evidence has been accumulating that inositol-containing glycans play an important role in signal transduction in various cells. It has been demonstrated that insulin stimulates the release of inositol phosphate glycans (IPG's) from the cell membrane of insulin-sensitive cells and that these soluble molecules possess insulin-like properties.[1] Similar experiments have suggested that interleukin-1,[2] interleukin-2,[3] and nerve growth factor[4] also result in IPG release in their target cells. However, considerable structural diversity of these IPG molecules has been reported, including the presence of D-*chiro*-inositol and D-galactosamine[5] vs. *myo*-inositol and D-glucosamine,[6] and D-galactose[7] vs. D-mannose.[5] As yet, no definitive relationship between structure and signalling capability has been established for the IPG's.[8] Accordingly, preparation of biologically active IPG's by unambiguous chemical synthesis is desirable to deconvolute the structural subtleties and elucidate the signalling pathway(s).

It has been established that the pentasaccharide VSG membrane anchor **2** derived from *Trypanosoma bruceii* (v118) by Pronase treatment has significant insulin-like activities in several intact cell assays,[9] probably due to its structural similarity with the IPG second messenger(s). It has also been found that synthetic *myo*-inositol-containing disaccharide cyclic phosphate **3**,[10] and related *chiro*-inositol-containing disaccharide phosphates[11] exhibit limited insulin-like activity. Accordingly, information regarding the biological activity of molecules spanning this structural range (i.e. di- to penta-saccharides) is of greatest interest. In this communication, we describe the chemical synthesis of D-mannosyl-(α1-4)-D-glucosaminyl-(α1-6)-D-*myo*-inositol-1:2-cyclic phosphate (**1**). This compound constitutes the terminal trisaccharide portion of the insulin-mimetic VSG membrane anchor **2** from *T.bruceii*.

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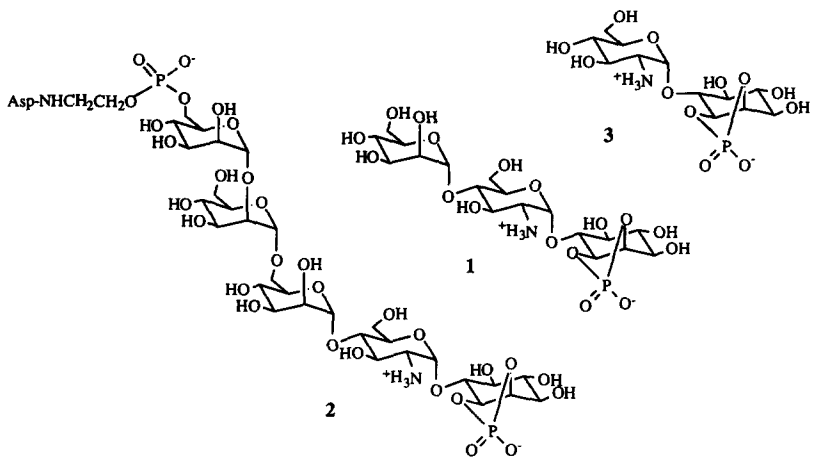

A suitably protected *myo*-inositol derivative was prepared from optically pure inositol **4**, available from D-xylose via a known sequence.[12] Treatment of **4** with N, N'-carbonyldiimidazole followed by removal of the silyl protecting group afforded the differentially protected inositol **5**,[13] suitable for glycosylation.

The glucosamine precursor, **6**, was prepared from tri-*O*-acetyl-D-glucal by the known procedure.[14] Glycosylation of **6** with methylthio mannoside **7**,[15] according to Ogawa's method,[16] provided α-disaccharide **8**[13] in 53% yield. Compound **8** was subjected to fluoride-promoted desilylation, converted to the glycosyl fluoride (DAST), and coupled with inositol **5** via Cp_2ZrCl_2-AgOTf activation[17] to produce trisaccharide **9**.[13]

Selective removal of the cyclic carbonate moiety of **9** followed by phosphorylation of the resulting diol with the reagent generated from $MeOPOCl_2$ and pyridine[18] produced the fully protected trisaccharide cyclic phosphate. Finally, removal of all benzyl protecting groups and reduction of the azido group was accomplished by treatment with sodium in liquid ammonia. The dissolving metal reduction reaction was quenched with NH_4Cl (s) at -78 °C followed by addition of MeOH at -78 °C and warmed to 20 °C. Desalting of **1** via Sephadex G-10 column chromatography (H_2O) yielded zwitterion **1**[13] (56% over 2 steps).

The biological properties of trisaccharide **1** and related compounds are currently being evaluated.

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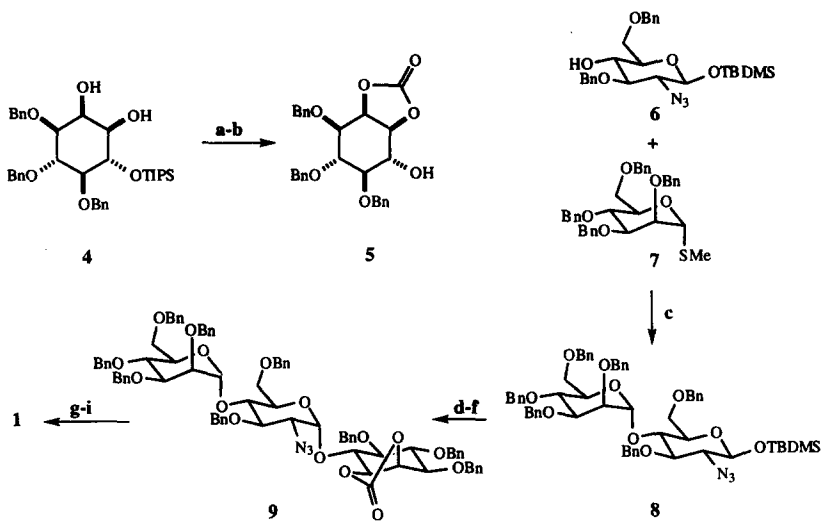

(a) N, N'-carbonyldiimidazole, benzene, 20 °C, 17h; (b) TBAF, THF, 0 °C, 3 min (84% over 2 steps); (c) PhSeCl, AgOTf, toluene, - 42 °C, 1 h (53%); (d) *n*-BuN_4F, HOAc, THF, 20 °C (84%); (e) DAST, THF, -42 °C, 30 min (90%); (f) **5**, Cp_2ZrCl_2, AgOTf, toluene, -42 °C to 20 °C, 5h (28% α; 26% β); (g) 1 M LiOH, THF, 20 °C, 12h, (80%); (h) $MeOPOCl_2$, pyridine; (i) Na, NH_3 (l), -78 °C, 15 min (56% over 2 steps).

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13. Spectral data for **5**: ^{1}H NMR ($CDCl_3$) δ 3.88 (dd, J=4.5, 10.7 Hz, 1H), 3.80 (dd, J=2.4, 4.5 Hz, 1H), 3.90 (ψt, J=2.8 Hz, 1H), 4.25-4.75 (m, 8H), 4.85 (dd, J=3.3, 8.7 Hz, 1H), 7.15-7.5 (m, 15H, phenyl). **8**: ^{1}H NMR ($CDCl_3$) δ 0.19 (s, 3H, CH_3), 0.20 (s, 3H, CH_3), 0.94 (s, 9H, *t*-Bu), 3.25 (dd, J=8.6, 9.9 Hz, 1H, H3 gln), 3.36-3.42 (m, 2H, H5 & H2 gln), 3.58 (dd, J=1.5, 9.1 Hz, 1H, H3 man), 3.64-3.83 (m, 7H), 3.98 (ψt, J=9.3 Hz, 1H, H4 man), 4.25 (d, J=12.2 Hz, 1H, 1/2 CH_2Ph), 4.35 (d, J=12.2 Hz, 1H, 1/2 CH_2Ph), 4.43-4.63 (m, 9H), 4.82 (d, J=10.8 Hz, 1H, 1/2 CH_2Ph), 4.94 (d, J=11.4 Hz, 1H, 1/2CH_2Ph), 5.30 (d, J=2.0 Hz, 1H, H1 man), 7.14-7.40 (m, 30H, Ph). ^{13}C NMR ($CDCl_3$) δ 100.15 (d, J=169 Hz, C1 man), 97.15 (d, J=160 Hz, C1 gln). **9**: ^{1}H NMR ($CDCl_3$) δ 3.39 (dd, J=3.6, 9.7 Hz, 1H, H2 gln), 3.51-3.90 (m, 12H), 4.02 (ψt, 2H), 4.20-4.66 (m, 17H), 4.79-5.02 (m, 4H), 5.27 (d, J=1.9 Hz, 1H, H1 man), 5.34 (d, J=3.6 Hz, 1H, H1 gln), 7-7.5 (45H, m, Ph). LR FAB (pos. ion mode): Found: 1388; Calculated for $C_{82}H_{83}N_3O_{16}$ + Na: 1388.57. **1**: ^{1}H NMR (D_2O) δ 3-4 (m, 16H), 4.35 (ddd, 1H, H1 inos), 4.5 (ψt, 1H, H2 inos), 5.1 (ψs, 1H), 5.3 (ψs, 1H). ^{31}P NMR (D_2O): 17.135 ppm (relative to 85% H_3PO_4). HR FAB MS (negative ion mode): Found: 564.1354; Calculated for $C_{18}H_{32}NO_{17}P$ - H: 564.1329.
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